

Microbiological Analysis of Stuffed Mussels Sold in the Streets

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Abstract Stuffed mussel is a traditional food that sold by street vendors in various countries. In the present study, samples of stuffed mussels were collected from various places in Ankara. The mussels were analyzed to show the microbiological risks for human health. Thirty samples (600 stuffed mussels in total) were collected periodically and microbiological analyses were performed by standard procedures for *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Clostridium* sp. In terms of *Salmonella* sp., approximately 50% of samples were not suitable for consumption. Besides, in accordance with Turkish Food Codex Microbiological Criteria Announcement in terms of *E. coli* 30%, in terms of *B. cereus* 80%, in terms of *S. aureus* 76.6%, in terms of *Clostridium perfringens* 13.3% of these samples were not suitable for consumption. The aim of this study is to discuss the microbiological quality of stuffed mussels as a ready-to-eat food according to Turkish Food Codex (TFC). The result of this investigation indicates that stuffed mussels as a street food may constitute a potential health hazard, depending on contamination level and lack of sanitary practices, and therefore, handling practices should require more attention and improvement.

Keywords Stuffed mussels · Microbiological analysis · *E. coli* · *Clostridium perfringens*

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Introduction

Stuffed mussel is a traditional food that is sold by street vendors in Turkey and other Mediterranean Sea countries. *Mytilus galloprovincialis* is called “black mussel” in Turkey and is used for stuffed mussel at most. Although there have been a lot of stuffed mussel recipe in some regions in the world, stuffed mussels are consumed by cooking differently in Turkey. In the recipe of stuffed mussels firstly, cockleshells are cleaned well by shaving and all featherlike structures are removed. Then, rice, oil, salt and mixed of spices is stuffed in the cockleshells. They also include mussel meat inside of them. Then, the cockleshells are closed firmly before they are cooked with steam. In recent years, stuffed mussel sale increased in a lot of food consumption places (restaurants, supermarkets, etc.) in Turkey [1].

The mussels, that are not stored in suitable conditions or not processed when they are alive, spoil. Therefore, they should be frozen when they are uncooked or after cooking [2, 3]. Mussels pump large quantities of water through their bodies, so they accumulate the toxic substances and microorganisms present in their environment [4]. In addition, their high glycogen and free amino-acid content, high water activity (aw. 0.95) and high pH (6.7–7.1) make them an ideal substrate for the growth of microorganisms. Therefore, they are at high risk of food poisoning when they are ingested [5, 6]. Besides, they indicate that if *E. coli* counts of uncooked mussels do not pass the 20% of total microbial load, these mussels can be processed. However, safety of these food sold in the streets is affected by a lot of factors. These factors vary from the quality of raw material to preparation by hands. These fast-food-like foods sold in the streets are exposed to environmental conditions (insects, flies, dust, etc.). Besides, some of the street

hawkers are not suitable for the safety food production such as cross contamination, keeping the foods in unhygienic conditions, exposing to inadequate time and heat [7–9].

Stuffed mussels caused various food borne diseases in the world as they have a lot of pathogenic bacteria [1]. Microorganisms and their values in ready for consumption daily foods and snacks that threaten health are given in Turkish Food Codex Microbiological Criteria Announcement (Announcement No: 2001/19). In such snacks *E. coli*, *Bacillus cereus* (CFU/g), *Staphylococcus aureus* (CFU/g), *Clostridium perfringens*, *Salmonella* spp. should be analyzed according to this Announcement. Values that should be found in suitable for Microbiological Criteria Announcement, ready for consumption foods and snacks are given in Table 1.

Pathogenic *E. coli* causes various diseases by its virulence factors. These virulence factors affect critical host cell processes such as protein synthesis, signal transduction, cytoskeleton function, cell division, ion release, transcription, apoptosis and mitochondrial functions [10]. *B. cereus* causes two different food poisonings: diarrhea type and emetic type. Diarrhea type food poisoning occurs with complex enterotoxins and these toxins are released by *B. cereus* during its vegetative growth in intestine. Emetic toxin is released by the cells growing in the food [11]. *C. perfringens* is distributed in large environments and usually presents in intestine of animals. It causes gangrene and gastrointestinal diseases (e.g. food poisoning and necrotic enteritis) [12]. Epidemiology of methicillin-resistant *S. aureus* infections is complex. Chronic live, lungs or vascular diseases in adults, long term treatment with dialyze, malignance or antimicrobial agents other risk factors also were identified [13]. *Salmonella enterica* presents in humans more than *S. typhimurium*. It was shown that the most extensive cause of the diseases is egg and the strains that cause outbreaks have found in egg. These strains cause Salmonellosis [14].

In this study, it was aimed to analyze the stuffed mussels sold in the streets in Ankara for microbiological quality.

Materials and Methods

Stuffed Mussel and Sample Collection

30 stuffed mussel samples were collected from various street venders in Ankara. 20 mussels were collected for each sample (Totally 600 stuffed mussels). These stuffed mussels were taken to laboratory in suitable conditions (on ice) and analyzed.

Microbiological Analysis

Three-tube MPN (Most Probable Number) method was applied for the detection of *E. coli* [1]. According to this method LSB (Lauryl Sulphate Broth, Oxoid) containing tubes were inoculated. After the incubation at 37°C for 24–48 h the tubes in which blurry and the gas formation in Durham tubes were observed, were accepted as positive. From these positive tubes it was inoculated into EC broth (*E. coli* Broth, Merck) containing tubes [15]. After the incubation at 44.5°C for 24 h it was inoculated into EMB Agar (Eosine Methylene Blue Agar) from positive tubes and the samples that were seemed in green highlights were considered as positive. MYP Agar (Mannitol Egg Yolk Polymixin)—Cereus Selective Agar—was used for *B. cereus* identification [16]. The dilutions from mussels were prepared to 10⁶ and from these dilutions they were inoculated to MYP Agar plates. After incubation at 37°C for 24 h the pink colonies that produce precipitation around them were counted.

0.1% peptone (Difco)-containing dilutions from mussel samples were prepared for *S. aureus* identification [17]. They were inoculated into BP (Baird Parker, Difco) Agar by spread plate method. After the incubation at 37°C for 24–48 h susceptible colonies were counted. One of the egg yolk positive, black colonies was inoculated into Brain Heart Infusion Broth for the certain result. After the incubation at 37°C for 18 h coagulase and thermonuclease activities were analyzed.

Table 1 Microbiological criteria announcement for ready for consumption daily foods and snacks

	n ^b	c ^c	m ^d	M ^e
<i>E. coli</i> ^a	5	2	<3	9
<i>Bacillus cereus</i> (Colony Forming Unit/gram)	5	1	1.0 × 10 ²	1.0 × 10 ³
<i>Staphylococcus aureus</i> (Colony Forming Unit/gram)	5	2	1.0 × 10 ¹	1.0 × 10 ²
<i>Clostridium perfringens</i> (Colony Forming Unit/gram)	5	2	1.0 × 10 ¹	1.0 × 10 ²
<i>Salmonella</i> sp.	5	0	Not found in 25 g	

^a According to MPN table, ^b sample number, ^c allowed sample numbers that have the value between m and M values, ^d reflects the upper limit of a good manufacturing practice (GMP), ^e marks the limit beyond which the level of contamination is hazardous or unacceptable

During identification of *Salmonella* for the pre-enrichment step 25 g mussels were put into 225 ml Buffered Peptone Water (BPW, CM509B Oxoid, Basingstoke, Hampshire, UK) and after homogenization in stomacher they were incubated at 37°C for 24 h [18]. After incubation 10 ml homogenized sample was inoculated into 90 ml Selenite Cystine Broth (SC, CM699B + LP121A Oxoid) and 0.1 ml from BPW was inoculated into 10 ml Rappaport–Vassiliadis medium (RV, CM669 Oxoid) and both were incubated at 37°C for 24 h. After incubation they were inoculated into Brilliant Green Agar (BGA, CM329B Oxoid) and incubated at 37°C for 24 h. Latex agglutination test was applied to susceptible colonies.

Three-tube MPN method was used for the identification of *C. perfringens* [19]. According to this method, 10 g mussels were homogenized in 90 ml BPW. The dilutions to 10³ were prepared. 1 ml from these dilutions was inoculated into 10 ml Differential Reinforced Clostridial Broth (DRCM; Merck Co.). After the incubation at 37°C for 24 h the tubes that produce black precipitation were accepted as positive.

Results and Discussion

The results of the microbiological analyses applied in this study are given in Table 2 and Fig. 1.

B. cereus, *S. aureus*, *E. coli*, *Salmonella* sp. and *C. perfringens* were positive in order 86.6 (520), 60 (360), 100 (600), 46.6 (280) and 100 (600) % of stuffed mussels. *S. aureus* is also considered the third most important cause of disease in the world amongst the reported food-borne illnesses [20]. It was reported as responsible for 25% of all food-borne illnesses in the US [21]. Presence of *S. aureus* is human sourced. It indicates the contact and cross contamination with hands [22]. In this study, *S. aureus* was detected in 360 (60%) stuffed mussel samples. The criteria for stuffed mussels according to Turkish Food Codex [23] is <10 CFU/g (satisfactory), 10–10² CFU/g (acceptable) and 10² < CFU/g (Unacceptable). It was detected that 460 (76.6%) samples of 600 stuffed mussels are unacceptable

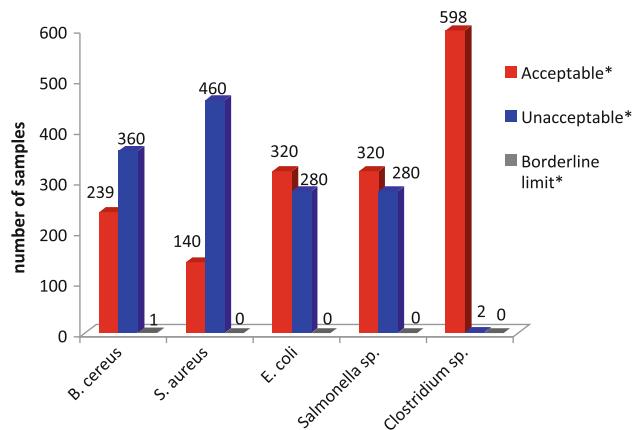


Fig. 1 The number of acceptable, unacceptable samples and borderline limits of samples according to the values given in Table 1. *The red columns indicate the number of samples that have the acceptable values according to Turkish Food Codex. The blue columns indicate the numbers of samples that have the unacceptable values according to Turkish Food Codex. The grey columns indicate the numbers of samples that have borderline limit values according to Turkish Food Codex (The values according to Turkish Food Codex are given in Table 1)

in our study. These results indicate the deficiency of personnel hygiene, use of protective utensils during processing (mask, gloves, bonnet, etc.). As we mentioned above, contamination risk is very high during the production process of stuffed mussels. Because there is no enough control for this process. The stuffed mussels sold in the streets are not controlled by relevant institutions. Therefore consumers are not able to know the conditions in which stuffed mussels cooked. Bingol and Colak [24] found that 27 of 168 stuffed mussel samples were unacceptable in their study. Our ratios are much more than their results. This can arise from the vendors or the conditions in that the stuffed mussels were sold.

B. cereus presents in nature a lot. Presence of it is risk for consumers via toxin producing that cause food poisoning [25]. *B. cereus* contamination causes gastrointestinal illness by several protein toxins and one heat stable peptide toxin called cereulide-causing emetic type of disease. *B. cereus* grows and produces emetic toxins in a

Table 2 The results of analysis

Microorganisms	Min (CFU/g)	Max (CFU/g)	Average (CFU/g)	n ^{pb}	Ratio of positive samples (%)
<i>B. cereus</i>	9.0 × 10 ¹	>3.0 × 10 ⁵	1.3 × 10 ⁵	520	86.6
<i>S. aureus</i>	2.6 × 10 ³	>2.9 × 10 ⁸	2.1 × 10 ⁷	360	60
<i>E. coli</i> ^a	<3.0	>2.4 × 10 ³	3.5 × 10 ²	600	100
<i>Salmonella</i> sp.	–	–	–	280	46.6
<i>Clostridium</i> sp.	<3.0	>2.4 × 10 ³	1.6 × 10 ²	600	100

^a According to the MPN table, ^b positive sample numbers. The table indicates the minimum (Min), maximum (Max) and Average results of 600 samples analyzed in our study for the given microorganisms

relatively short time on cooked rice and other starchy foods stored at room temperature [26, 27]. *S. aureus* and *B. cereus* counts were high because stuffed mussels are kept in the streets for a long time although they were cooked. Cooking rice with vegetables causes a very good condition for spore forming bacteria. *B. cereus* is also spore forming bacteria. So that it can resist to unsuitable conditions during cooking. *S. aureus* contamination can be occurred as the mussels would be prepared by hand. *B. cereus*, *S. aureus*, *Salmonella* sp. in order of 13.3, 23.3, and 53.3% of mussels were not detected. Besides, *E. coli* and *C. perfringens* in order of 60 and 73.3% of mussels were found as <3.0 CFU/g.

Coliform bacteria, which are widely dispersed in soils, plants, surface waters, and the gut of warm-blooded animals, can influence food safety and preservation because these organisms are an indicator of fecal contamination [28]. The detection of coliforms is widely used as a means of measuring the effectiveness of sanitation programs, since their presence indicates a substantially increased risk of the presence of pathogens [29]. Coliforms include *E. coli* and the presence of *E. coli* in stuffed mussels is a matter of concern because some strains may be pathogenic or can be propagated with other pathogenic organisms [24]. The *E. coli* pathotypes have been associated with cases of mild and severe diarrhea in adults and children, mostly in developing countries [30].

In this study *E. coli* was found positive in 100% of stuffed mussel samples. This indicates that poor hygiene and cross-contamination during cooking or from outside. 280 (46.6%) of samples are unacceptable to consume according to TFC in our study. Bingol and Colak [24] analyzed stuffed mussels in Istanbul and 31 (18.4%) of 168 samples were unacceptable in their study. The differences between these studies may arise from our samples. Our samples are collected from only street sellers. In their study, the samples were collected from restaurants, buffets and street sellers.

The results of our investigation confirm that the samples sold in streets are not suitable to consume due to their microbiological quality. Also TFC limits can be considered. Insensibly sale of the foods that have pathogens in such high values in streets can be very dangerous. Necessary precautions should be taken such as hygiene and sanitation education, etc. Also according to the *WHO Principles for the Establishment and Application of Microbiological Criteria for Foods CAC/GL 21—1997*, limits used in criteria should be based on microbiological data appropriate to the food and should be applicable to a variety of similar products. They should therefore be based on data gathered at various production establishments operating under Good Hygienic Practices and applying the HACCP system. Microbiological limits should take into

consideration the risk associated with the microorganisms, and the conditions under which the food is expected to be handled and consumed. Necessary precautions should be taken such as hygiene and sanitation education to prevent the contamination during production in terms of limiting the microorganism counts according to Turkish Food Codex. In this regard gloves should be worn during the cleaning of cockleshells well by shaving and removing all featherlike structures. The ingredients should be suitable for use in foods. After cooking the storing conditions should be set well.

Conclusions

Ready-to-eat foods such as stuffed mussels sold in the open areas without any precautions could be a major cause of food-poisoning and foodborne diseases. The results show us that better control is needed and that the cooking, distributing conditions should be controlled to enhance the microbiological safety of these foods.

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